

# **TOWARDS STUNT-FREE LONREN RENIFORM NEMATODE RESISTANCE BY DISSECTION OF AN ALIEN R-GENE CHROMOSOME SEGMENT AND DETECTION OF SIGNIFICANT FUNGAL INTERACTIONS**

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## **Abstract**

Reniform (*Rotylenchulus reniformis*) nematodes cost US cotton growers over 130 million dollars per year. Genetic resistance of the wild African species *Gossypium longicalyx* to reniform nematodes was previously transferred into upland cotton (*Gossypium hirsutum* L.). The responsible gene was dubbed *Ren<sup>lon</sup>*. LONREN and other cotton lines containing *Ren<sup>lon</sup>* are highly nematode-resistant, but the seedlings and plants were observed to be "stunted" when grown in nematode-infested fields. Hypothesized causes include hypersensitivity reaction per se, or increased susceptibility to soil pathogens. The objectives of my research have been [1] to develop *Ren<sup>lon</sup>*-linked markers and map the *Ren<sup>lon</sup>* region at high resolution, [2] to recombine *Ren<sup>lon</sup>* and nearby loci and determine if "stunting" is due to linkage drag, and [3] to create genomic and germplasm resources for genetic manipulation of chromosome-11, especially *Ren<sup>lon</sup>*.

To develop genomics resources and explore the possibility of separating *Ren<sup>lon</sup>* from linked factors that hypothetically might cause "stunting", thousands of putative SNPs identified between *G. hirsutum* and *G. longicalyx* by RNA-seq were collaboratively aligned to the D5 reference genome, particularly the Scaffold\_7 region homeologous to the *Ren<sup>lon</sup>* region. Putative SNPs from this region were screened against a genetic screening panel, and 85 SNP markers were identified as *Ren<sup>lon</sup>*-linked. We established an inexpensive method for high-throughput non-destructive seed DNA extraction for PCR-based genotyping, and subsequently used it for high-resolution mapping of 10 SNPs in the proximal alien segment near *Ren<sup>lon</sup>*. Seventeen thousand six hundred BC<sub>1</sub>F<sub>1</sub> seeds were screened by marker-assisted selection with the two closest *Ren<sup>lon</sup>*-linked SNP markers to identify recombinants. Five BC<sub>1</sub>F<sub>1</sub> recombinants were identified, from which two of them were free of "stunting" as determined by stunting assays. Reniform resistance tests indicated that both of them lost resistance to reniform. Resistance and stunting were not separated. If correct, the results suggest that stunting of LONREN seedlings is not due to linkage drag, or we did not have enough recombinants to recover the desired individuals that separate the resistance and stunting. Growth chamber experiments indicated that the seedling "stunting" of *Ren<sup>lon</sup>* plants depends more heavily on *Thielaviopsis* root rot in cotton and its interactions, whereas the nematodes *per se* have little or no effect.